

Award Number: W81XWH-12-1-0168

TITLE: Imaging Prostatic Lipids to Distinguish Aggressive Prostate Cancer

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REPORT DATE: December 2016

TYPE OF REPORT: FINAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
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1. REPORT DATE Dec-2016		2. REPORT TYPE FINAL		3. DATES COVERED 8Aug2012- 7Aug2016	
4. TITLE AND SUBTITLE Imaging Prostatic Lipids to Distinguish Aggressive Prostate Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-12-1-0168	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Jackilen Shannon, PhD E-Mail: shannoja@ohsu.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Oregon Health & Science University 3181 SW Sam Jackson Park Road Portland, OR 97206				8. PERFORMING ORGANIZATION REPORT NUMBER 1	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT <p>Objectives: In this application, we propose to build upon our current work to determine the association between fatty acid synthase (FAS) overexpression and intraprostatic fat as measured by in-vivo imaging using proton magnetic resonance spectroscopy imaging in the prediction of prostate disease aggressiveness. Mechanisms linking fatty acid synthase overexpression, lipid accumulation, lipid oxidation, and tumor aggressiveness will be explored using metabolomics. Plan: Employing a cross-sectional design we will recruit 50 men with low-grade and 50 men with high grade prostate cancer post-diagnosis as determined prior to prostatectomy. Each patient will complete one proton magnetic resonance spectroscopy imaging session and provide access to his prostatectomy tissue.</p> <p>Study aims: Among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by Gleason scoring) we propose to: 1) Determine the correlation between FAS expression in prostatectomy samples and the amount of intraprostatic lipid using ¹H magnetic resonance spectroscopic imaging (proton MRSI) with an endorectal coil. 2) Identify the association between FAS expression and FAS activity in prostatectomy samples, intraprostatic lipid as measured by MRSI and prostate tumor aggressiveness. 3) To quantify key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in the prostatectomy samples.</p>					
15. SUBJECT TERMS Nothing listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	20	19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION: Mounting evidence suggests that dysregulation of fatty acid synthase (FAS), the rate limiting multienzyme in the de novo formation of free fatty acids, is an early and important step in carcinogenesis and transformation to aggressive prostate cancer. Excess production of free fatty acids by FAS occurs through enhanced synthesis of malonyl-coA from acetyl-CoA and leads to increased cellular triglyceride formation and deposition. Thus we **hypothesize** that increased intraprostatic lipid concentration as measured by ¹H Magnetic Resonance Spectroscopy (MRSI) will identify tissues with higher FAS activity, which in turn will be those that exhibit more aggressive disease. In more aggressive cancer tissues, we expect to find metabolic signatures of enhanced fatty acid oxidation. In showing an association between FAS protein overexpression by histology, in-vivo intraprostatic fat as measured by ¹H MRSI, metabolic signatures of lipid oxidation and metabolism, and prostate cancer aggressiveness, our **objective** is to provide support for the novel application of this imaging modality for use in the clinical setting to determine the proper management of newly diagnosed prostate cancer. Specifically, among men diagnosed with low grade (proposed as more indolent) and high grade (proposed as more aggressive) prostate cancer (as determined by the 2011 National Comprehensive Cancer Network (NCCN) guidelines [6]) we propose to 1) determine the correlation between the amount of intraprostatic lipid using 1H magnetic resonance spectroscopic imaging (MRSI) with an endorectal coil obtained prior to prostatectomy with FAS protein expression measured in benign and cancer tissue from prostatectomy samples; 2) identify the association between FAS protein expression in prostatectomy samples, intraprostatic lipid as measured by 1H MRSI, and prostate tumor aggressiveness; and 3) quantify the association between key metabolic intermediates involved in lipid metabolism, mitochondrial function, inflammation, and apoptosis in prostatectomy samples and FAS protein expression, intraprostatic lipid and tumor aggressiveness.

BODY: Department of Defense funding to allow initiation of this project was set up and received locally at the end of January/ beginning of February, 2013. From that point forward we have made progress in meeting the following items from our statement of work as described below (full SOW attached).

Task 1. Finalize clinical protocol and training (Shannon & Purnell) Months 1-6

1. Develop tracking system for recording patient recruitment, contact and consent information; laboratory and specimen receipt and analysis. (Shannon)

This task has been completed. All patient records are recorded using the Progeny system. Security is assured by maintaining all identifiers on the VA computer system with a crosswalk to a random unique ID maintained in the tracking program.

2. Obtain IRB approval from Portland Veterans Affairs Medical Center (PVAMC) (Shannon) and Oregon Health & Science University (OHSU) (Purnell)

*This task has been completed. Portland VA Medical Center (PVAMC) IRB approval was received 9/9/2012. Oregon Health & Science University (OHSU) IRB approval was received on 12/28/2012. **2014 Update:** We initiated a move to the joint IRB (PVAMC+OHSU) in order to streamline all project modifications, assure we operated under the same protocol at all times as well as to minimize paperwork submission on 9/3/2013; our move was approved on 10/31/2013. **2015 Update:** JOINT IRB approval remains intact. **2016 Update:** JOINT IRB approval remains intact. This study is closed to recruitment and open for data analysis.*

3. Finalize and review services with Clinical and Translational Research Center (CTRC) bionutrition staff. (Shannon & Purnell)

We are not utilizing the CTRC bionutrition staff at this time. We are, however, utilizing the CTRC core laboratory to process our urine and blood specimens since the first subject's enrollment onto this study on March 8th, 2013. This SOW point has been modified to reflect this change in our study plan.

4. Arrange meetings between research coordinator and Advanced Imaging Research Center staff in order to:
(Purnell)
 - a. Identify point of contact cascade
 - b. Collaboratively develop study-specific Standard Operating Procedures
 - c. Train all staff on following research protocol exclusively
 - d. Gather regulatory documents

*These tasks have been completed. Monthly research meetings are held with all study staff and the interdisciplinary investigational team. Point of contact has been identified for each step in the research process and an SOP has been developed for consistent recruitment of subjects and exchange of data from the urologist to the research coordinator to the MRSI technician and investigator to the pathologist (see Appendix 2; biopsy and MRSI measurement report form). Research protocol training has been completed with Ms. Farris and Mr. Stoller as well as all participating investigators. All regulatory documents are stored per VA protocol. **2014 Update:** As our clinical radiologist, Dr. Fergus Coakley, has emphasized, the accomplishment of assembling a multi-disciplinary team to meet on a monthly basis is of great import. We discuss the research process, its progress, provide quality improvement and care as well as maintain all aspects of the study as a cohesive group. Communication is clear, consistent and concise.*

We have new, supporting coordination staff; one recruiting participants from OHSU (Ms. Martinez), another from PVAMC (Ms. Palma). The addition of staff has increased our capacity for assuring subjects have project staff with them/ in the vicinity for the research visit at all times. Our coordinating staff assures the post-procedure handout for complications is provided, and is a reliable escort through campus and the imaging facility back to familiar ground. Additional procedures have been identified, streamlined and instituted over the course of the past year both at our monthly meetings as well as with subgroups intimately involved with subjects. This includes such procedures as 1) enemas must be completed a full hour prior to research MRSI with endorectal probe, 2) scheduling each subjects' imaging research visit for a full 2 hours in order to accommodate multiple steps in support of image capture process, 3) taking pictures of the prostate at the time of pathology processing and 4) assuring clinical radiology interpretation not only goes back to the imaging investigators but to the respective urologic surgeon as well. All changes to procedure were reviewed and approved by the IRB prior to implementation.

2015 Update: During more than one of our monthly, multi-disciplinary team meetings, we decided that it would be important to request a No-Cost Extension for this project.

2016 Update: Research meetings of all study staff and the interdisciplinary investigational team have continued. Since the end of all study tasks in January, meetings focus on final data analysis.

5. Review protocol and procedures with clinical staff; establish pathology residents' formal independent contracts (Shannon)

*Review of protocol and procedures has been an ongoing monthly task. Optimization of procedures has been ongoing and we have recruited 10 men whose data will only be utilized during this optimization time period. Independent contracts with the pathology residents occurred during the months of March and April 2013. **2014 Update:** Since our project's initial review and first DOD annual report, we have submitted 5 modifications and 1 continuing review. Please see 'Key Research Accomplishments' section for a full list and related descriptions. **2015 Update:** In the previous year, we submitted 3 modifications and 1 continuing review. Please see 'Key Research Accomplishments' section for a full list and related descriptions. **2016 Update:** In the previous year, no modifications were submitted; the study expires on February 1, 2017. A continuing review will be submitted to keep the study open for final analyses. Please see 'Key Research Accomplishments' section for a full list and related descriptions.*

Task 2. Initiate subject recruitment and testing Months 6-30

1. Identify potentially eligible patients, contact men and initiate recruitment (Shannon)
2. Complete consenting process and confirm eligibility for interested men (Shannon)

3. Conduct fasting blood collection, magnetic resonance spectroscopy imaging (MRSI) visits and prostatectomy tissue processing (**Purnell**)

Progress towards completion of these tasks is ongoing; as of September 01, 2015, we have enrolled and consented a total of 51 men to the study. Since our last year's annual progress report, 20 men successfully completed an MRSI with no complications. We have three pending MRSIs, one withdrawal and six screen fails (that is, no successful MRSI). We have collected specimens on 42 men. At the time of our 2014 annual report, we estimated an average recruitment rate from OHSU of 2 men per month; in the past year, all of our 20 men were recruited from OHSU. Despite this fact, project recruitment has not reached the total level we hoped and planned for. However, it is notable that we were not able to begin our project until we received funding from the DOD, which did not occur until 6 months post-award notification. Therefore, we have submitted a request for a 1-year No-Cost Extension in order to recruit an additional 10 men to the project.

We presented one technical abstract at this year's International Society for Magnetic Resonance in Medicine annual meeting (1). During which, we reported quantitative correction for intraprostatic MRSI lipid spectra. It is well known that periprostatic lipid content is significantly higher than that from inside the prostate gland. Even though different, advanced techniques have been proposed to minimize the signal contamination from periprostatic lipid to intraprostatic lipid spectra, a contamination free approach is yet to be identified. In fact, our results indicate that the contamination from clinically-available sequences can be so great that it could render proton MR spectroscopy near the vicinity of lipid peak, which includes that for lactate, uninformative. This is because the function peaks, e.g., lactate, often have more than three magnitude weaker MR spectroscopy signals. Thus, a very small fraction of residual lipid peak can still be significantly larger than that of lactate. Based on this finding, we added up to six single voxel spectroscopy (SVS) data acquisitions into our MRSI data acquisition protocol in 2015. Most SVS voxel locations are within the respective MRSI slices, so a direct comparison of MRSI signal to the SVS signal is potentially feasible. In addition and in order to facilitate co-registration between MRSI slices and histological tissue slice, the IRB approved in vivo inking of the prostate just prior to prostatectomy. As stated in the 2015 IRB and Research Updates, below. The full research team is in the midst of compiling all variables in support of interim – and eventually, final – data analyses.

Recruitment for this study was completed in November 2015; final study-related data collection was completed in January 2016. A total of 68 men were enrolled onto the study; 10 men's data were only utilized for optimizing study procedures, the remaining 58 (if not withdrawals [W] or screen fails [SF]) men are in the category whose data will be used in final analyses.

4. Compensate men for their participation in study (**Shannon & Purnell**)

*Within a month of a man participating on our trial, they have either been compensated for a successful MRI and/or travel reimbursement. **2104 Update**; this task is ongoing and will continue until recruitment is complete. **2015 Update**; compensating men for their participation is an ongoing, important task that will continue until the end of the project (into the requested No-Cost Extension year). **2016 Update**; all subjects have been compensated for their participation.*

Task 4. Conduct immunohistochemistry analyses (Shannon) Months 12, 24, 32 (3 batches)

Dr. George Thomas requested that we submit prostatectomy specimens for immunohistochemistry analyses (fatty acid synthase staining) throughout the life of the project v. in large batches. Therefore, Dr. Thomas and Mr. Stoller have been meeting regularly to read research slides, submit specimens for cutting and staining and track Dr. Thomas' scoring, all in support of eventual alignment with all other variables and, ultimately, data analyses.

Task 5. Conduct metabolomics analyses (Purnell) Month 30-34

A subcontract for metabolomics analyses is in place with Drs. Arun Sreekumar and Nagireddy Putluri at Baylor Medical School. All tissue collected to date has been shipped to Dr. Sreekumar's metabolomics team.

Baylor investigators will utilize our first 10 [test] subjects' specimens in order to optimize the quantification of fatty acid synthase expression and intraprostatic lipid accumulation in prostate cancer process but this data will not be used in final analyses. Additional specimens will be sent at the time of final subject recruitment.

Lipidomics Profiling of Case control samples

As described above, we have complemented lipidomics data for first test subjects using high resolution triple of mass spectrometry. The samples were extracted using newly established protocol. We have spiked the equimolar 10uL (from 100pmol/uL stock) internal standard containing all lipids (17:0LPC, 17:0PC, 17:0PE, 17:0PG, 17:0 ceramide, 17:0SM, 17:0PS, 17:0PA, 17:0TG 17:0MG17:0 ChoE, D31 TAG and D5- DAG) Mix and 20uL 17:0PI (LM-1802), mix well. After extracted the samples were reconstitute with 100ul buffer B (85%IPA+5%H2O+10%ACN) and inject 5uL (both positive and negative mode).

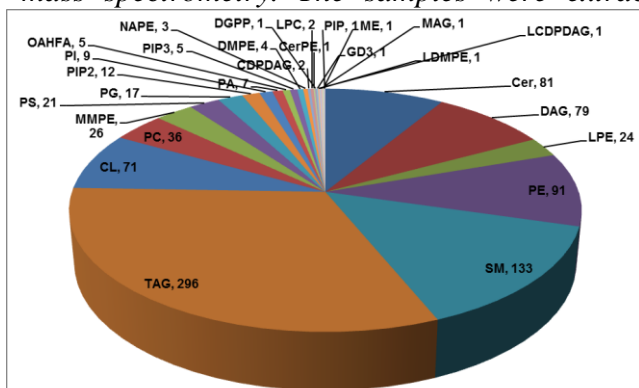


Figure 1. Identified lipids.

have quantified 500 lipids across our first 10 [test] subjects' specimens (See Table 1 for class of lipids). The pie chart showing the % of each class of lipids, those were identified in the test subjects. These CVs are calculated as SD/Mean with the natural log transformed data. The values are much lower than the ones calculated with the original dataset because of the transformation. Total 222 lipids having less than 20% of CV across all the test samples.

Table 1. Showing the identified lipids in test controls.

Class of lipid	No of Lipids	Method
Cer	81	Positive mode
DAG	79	Positive mode
LPE	20	Positive mode
PE	48	Positive mode
SM	123	Positive mode
TAG	296	Positive mode
CL	71	Negative Mode
PE	43	Negative Mode
PC	36	Negative Mode
MMPE	26	Negative Mode
PS	21	Negative Mode
PG	17	Negative Mode
PIP2	12	Negative Mode
SM	10	Negative Mode
PI	9	Negative Mode
PA	7	Negative Mode
OAHFA	5	Negative Mode
PIP3	5	Negative Mode
DMPE	4	Negative Mode
LPE	4	Negative Mode
NAPE	3	Negative Mode
CDPDAG	2	Negative Mode
LPC	2	Negative Mode
CerPE	1	Negative Mode
DGPP	1	Negative Mode
GD3	1	Negative Mode
LCDPDAG	1	Negative Mode
LDMPE	1	Negative Mode
MAG	1	Negative Mode
ME	1	Negative Mode
PIP	1	Negative Mode

Key Metabolomics Research Accomplishments

- 1) Developed unbiased mass spectrometry methods to profile 500 lipids.
- 2) Completed lipidomics analysis of 9 test subjects.
- 3) Defined distinct class of lipids in tissue samples.

Metabolomics Research Conclusions; we have defined tissue-associated lipidomics profiles.

Task 6. Final Analyses and Report Writing (Shannon & Purnell) Months 30-36

1. Final statistical analysis of data from immunohistochemistry, MRSI and metabolomics will be performed. ([SEE REPORTABLE OUTCOMES, below; page 7](#))
2. Prepare final report and initial manuscripts.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

Year 01 – 2013

- Portland VA Medical Center (PVAMC) IRB approval as of 9/9/2012.
- Standing monthly investigational team meeting initiated 11/8/2012.

- Added Medical Monitor, Arthur Hung, MD to project 11/14/2012.
- Oregon Health & Science University (OHSU) IRB approval as of 12/28/2012.
- Initiation of enrollment; first participant consented to study 2/22/2013.
- Continuing review PVAMC IRB approval 3/12/2013.
- Modification to add safety ocular x-ray to study; PVAMC IRB approval 3/29/2013.
- Modification to add safety ocular x-ray to study; OHSU IRB approval 4/28/2013.
- Dr. Fergus Coakley, OHSU Diagnostic Radiology Chair agrees to collaborate, consult and share his MRI in prostate cancer expertise with the investigational team, 5/20/2013.
- Modification to exclude recently-prescribed statin users (i.e.: on statin drug for less than 6 months) from study, increase to number of men (to 140); PVAMC IRB approval 6/18/2013.
- As of August 29, 2013, 9 men consented to study; 1 pending MRSI, 6 successful MRSIs, 2 screen fails.

Year 02 - 2014 IRB and Research Updates

- As of last year's report, we have enrolled 30 additional men to this study. Our veteran participants number 12, OHSU's are 18; please note that the first 10 subjects will not be included in any data analyses (the first 10 subjects acted as our 'test' subjects while we optimized our research project's multi-level, multi-resource, multi-system processes). We have a total of 24 successful (and analyzable) MRSIs, 1 pending MRSI, 3 screen fails and 1 withdrawal.
- Based on the optimization work, a standardized protocol for imaging, obtaining and processing tissue and obtaining and storing biologic specimens was put into place and recruitment into the primary study began 11/22/2013.
- All scientific investigators, study staff and clinical investigators continued to meet monthly to discuss study progress, necessary changes and review the timeline for study analyses.
- PVAMC and OHSU IRBs joined forces to provide researchers with one system for human subjects review and monitoring for projects that operate at both institutions. We submitted our modification to move the whole project to the 'joint' IRB as well as reconcile any remaining protocol differences; receiving JOINT IRB approval on 10/31/2013.
- Dr. Christopher Amling, OHSU Department of Urology Chair and surgical urologist agrees to collaborate and act as an addition recruitment site. Modification to add OHSU Department of Urology personnel (NP and Coord.) to the study; JOINT IRB approval on 11/7/2013.
- Due to confusion about the correct date to follow for continuing review, materials were submitted late for continuing review and the IRB approval lapsed for 3 weeks. All study activities were halted during this short time, regaining IRB approval on 1/21/2014.
- Dr. Mark Garzotto's conflict of interest form was incomplete and he was hence removed from the continuing review submission in order to rapidly re-gain IRB approval. Dr. Garzotto was re-added to the project on 3/24/2014.
- In order to communicate clearly and effectively with research participants, an enema instruction sheet was developed and approved by the JOINT IRB on 5/23/2014.

- At the time of the 2014 annual report update, a modification to add another OHSU Department of Urology clinician and update our post-MRI procedure handout is currently under JOINT IRB review.

Year 03 - 2015 IRB and Research Updates

- Mr. Wesley Stoller's name was added to our post-study visit handout in order to help triage any post-probe complications. We also added Dr. Theresa Koppie to the project, gaining JOINT IRB approval on 9/9/2014.
- Due to concerns raised by the OHSU pathology department over the availability of diagnostic tissue in men consented onto this study with small-focal tumor, we submitted a protocol modification at the time of our continuing review to narrow the eligibility criteria to men without singular small-focal tumor. In addition to this modification, we submitted paperwork to mark anatomical features of the prostate *in vivo* in order to better align the prostate with our MRI images during gross examination; one reviewing entity pushed back to remove this addition from our annual review paperwork. These modifications significantly delayed the continuing review submission. Therefore, our approval lapsed due to these issues; all study activities were halted during this short time, regaining JOINT IRB approval on 2/3/2015.
- We proposed inking the in vivo prostate in men (VA patients only) already sedated in preparation for prostatectomy. A second consent form for VA patients was approved. To date, we've had no VA patient consent to this research only process. We gained JOINT IRB approval for this option on 5/18/2015.
- Dr. Theresa Koppie left OHSU and was removed from the project; the JOINT IRB approved this personnel removal submission on 5/28/2015.

Year 04 - 2016 IRB and Research Updates

- Continuing Review with joint eIRB was approved 2/2/2016 (no modifications were submitted for this review).
- Completion of data analyses

REPORTABLE OUTCOMES: *Final Data Summary and Results 2016*

Team members Dr. Xin Li (imaging), Dr. George Thomas (pathology) and Dr. Motomi Mori (biostatistician) have compiled all current variables in preparation for statistical analysis of the immunohistochemistry and imaging measurements.

Data Sources

An analytical data set was created by merging fatty acid synthase (FAS) data and Gleason data by Subject ID. Because multiple single voxel spectroscopy (SVS) measures were made for each subject, the average SVS for tumor lesions and for normal lesions were calculated, then merged with FAS-Gleason data by Subject ID. The resulting analytical data set contains 58 subjects.

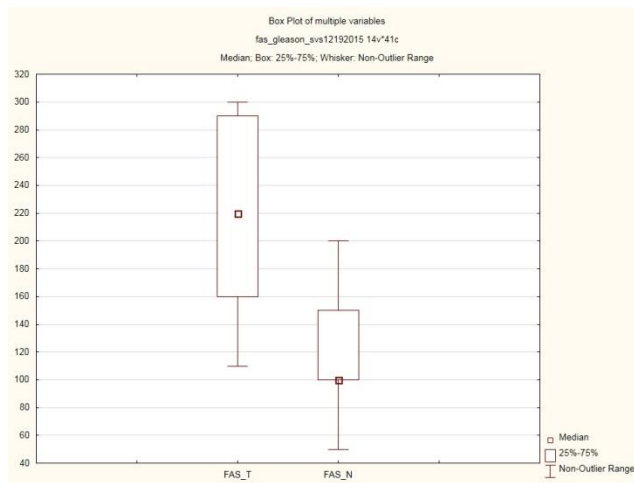


Figure 2. Box-plot of FAS protein expression in tumor (FAS_T) and normal (FAS_N) tissues

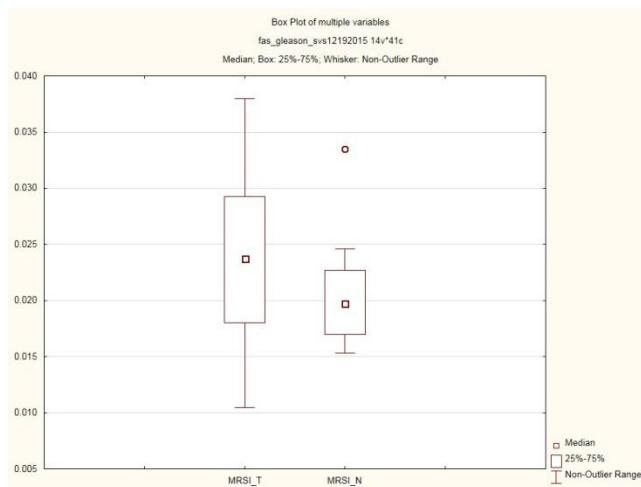


Figure 3. Box-plot of MRSI intraprostatic lipid in tumor (MRSI_T) and normal (MRSI_N) lesions

The target sample size was 100 subjects, 70 subjects with less aggressive (Gleason 3+4 or lower) prostate cancer and 30 subjects with aggressive prostate cancer (Gleason 4+3 or higher). At the time of study analyses (June 1, 2016), we identified 58 subjects who contributed to a varying number of endpoints (Table 2).

Table 2. Final data available			
Variable	Tissue Type	N (% of 58)	Note
Path Gleason Score	Tumor	53 (91%)	1 subject no RRP 4 subjects missing Path Gleason
FAS	Tumor Normal Tumor-Normal Pairs	25 (43%) 25 (43%) 22 (38%)	
MRSI (SVS)	Tumor Normal Tumor-Normal Pairs	29 (50%) 29 (50%) 29 (50%)	
FAS and MRSI	Tumor Normal	10 (17%) 11 (19%)	
Metabolomics Data	Tumor Normal Tumor-Normal Pairs	24 (55%) 32 (55%) 19 (33%)	2 samples from 5 subjects 2 samples from 10 subjects

Among 58 subjects, 5 subjects had missing path Gleason score (including one subject without radical prostatectomy), 31 subjects with low Gleason score (3+3, 3+4), and 22 subjects with high Gleason score. Table

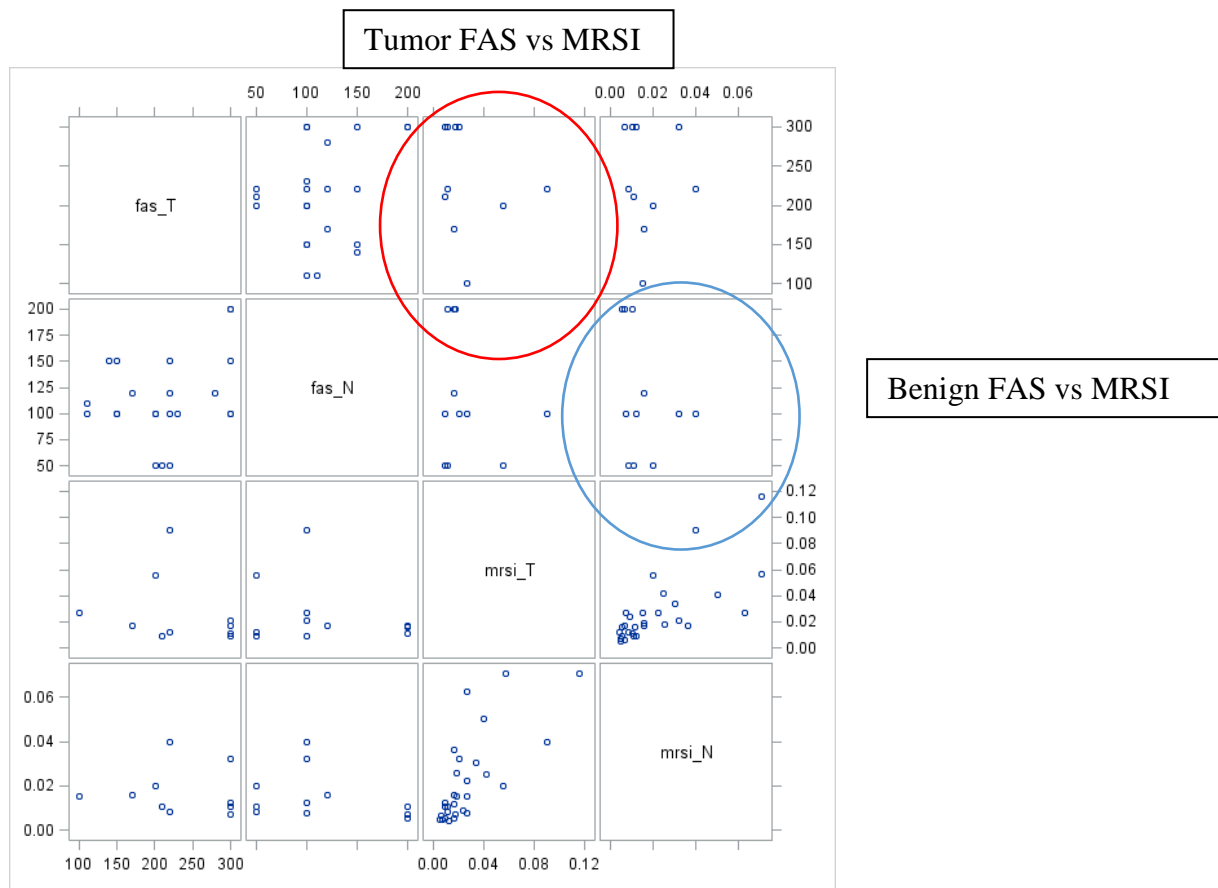
2 describes available data for key endpoints. Among 53 subjects with Path Gleason score, 58% was considered low Gleason (less aggressive tumors), while 42% was considered high Gleason (aggressive tumors). Because the study required prostatectomy and most patients with a Gleason score of less than 3+3 are not recommended for surgery.

Aim 1: Association between FAS vs. MRSI in benign and tumor tissues

Descriptive statistics for FAS and MRSI are presented in Table 3. Spearman correlation analyses were performed to assess the association among FAS tumor, FAS benign, MRSI tumor and MRSI benign tissues (Table 4). **There was no significant association between FAS vs. MRSI in tumors (Spearman $\rho = -.35$, $p = .32$, $N = 10$) or in benign tissues (Spearman $\rho = -.41$, $p = .21$, $N = 11$).**

Table 3. Descriptive statistics of FAS and MRSI variables				
Variable	N	Mean	ST. DEV.	Range
FAS Tumor	2	203.2	66.8	100-300
FAS Benign	2	116.8	41.7	50-200
MRSI Tumor	2	0.027	0.025	0.005-0.116
MRSI Benign	2	0.022	0.020	0.004-0.071

Table 4. Spearman correlation coefficients among FAS tumor, FAS benign, MRSI tumor and MRSI benign tissues			
	FAS Benign	MRSI Tumor	MRSI Benign
FAS Tumor	0.19 ($p = .40$; $N = 22$)	-0.35 ($p = .32$; $N = 10$)	-0.29 ($p = .41$; $N = 10$)
FAS Benign		-0.06 ($p = .86$; $N = 11$)	-0.41 ($p = .21$; $N = 11$)
MRSI Tumor			0.78 ($p < .0001$; $N = 29$)

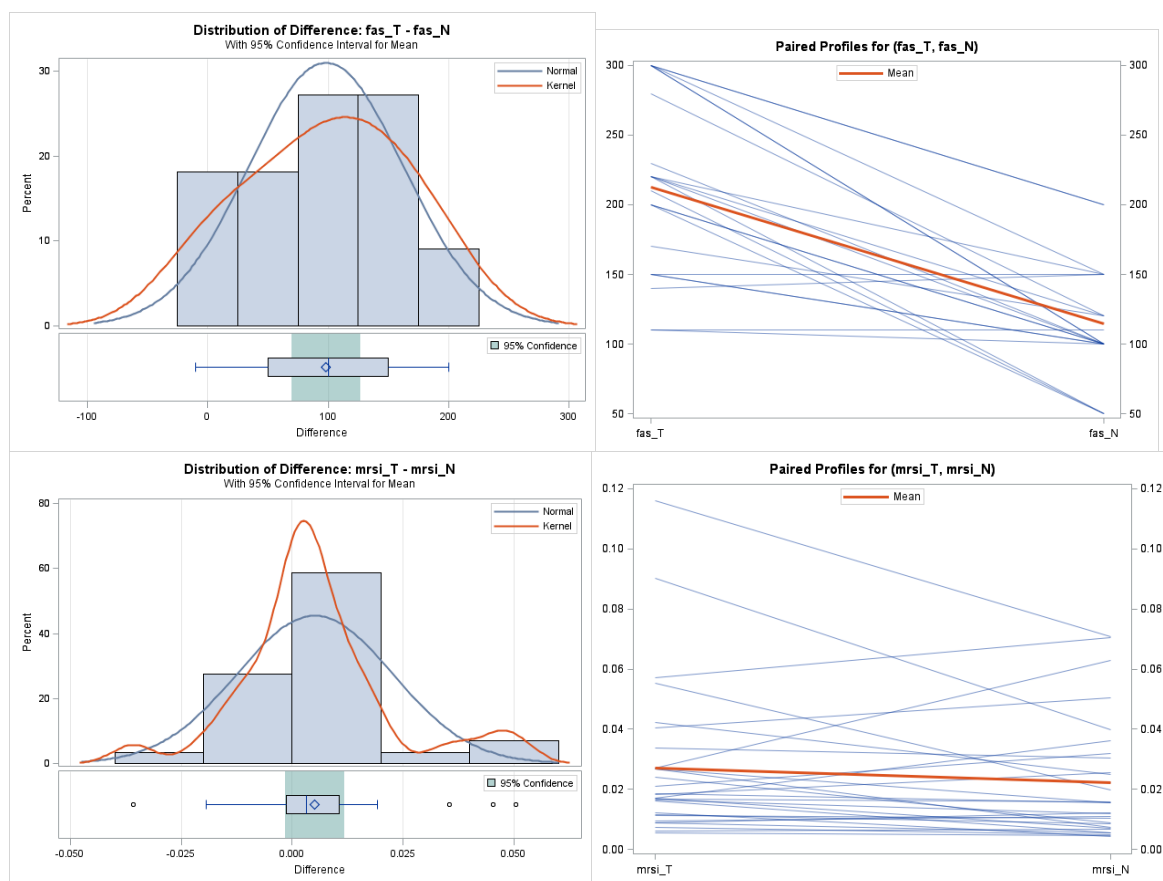


Aim 2: Comparison of FAS and MRSI between tumors vs. benign tissues and high vs. low Gleason tumors

A paired t-test was performed to evaluate differences in FAS between tumors vs. benign tissues and differences in MRSI between tumors vs. benign tissues. **FAS was significantly higher in tumors than benign tissues ($p < .0001$), while MRSI was not significantly different between tumors and benign tissues ($p = .1294$).**

Table 5. Comparison FAS and MRSI between tumors vs. benign tissues

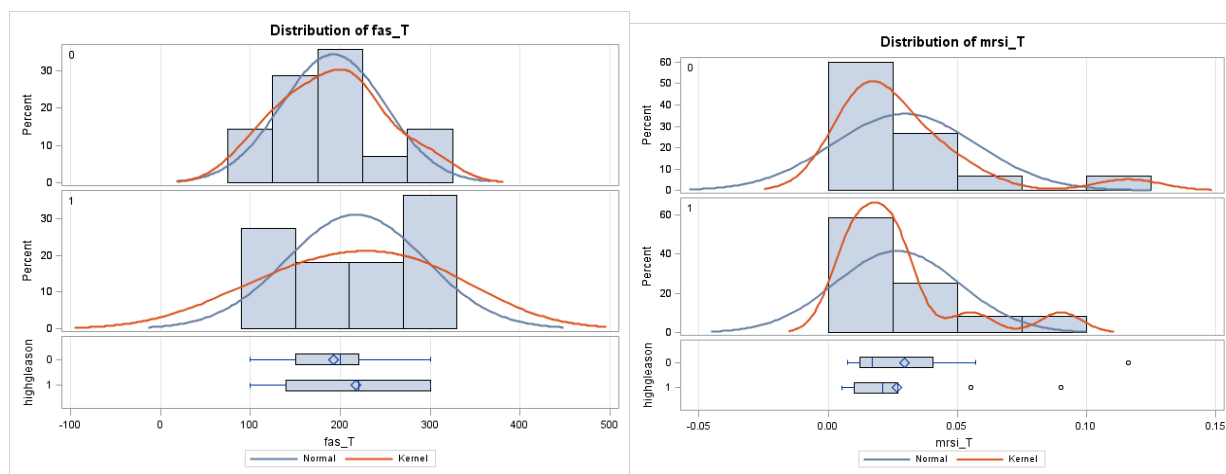
Comparison	N	Mean Difference	SE	95% CI of Mean	P value
FAS Tumor vs Benign	22	98.2	13.7	(69.7-126.7)	<.0001
MRSI Tumor vs Benign	29	0.005	0.003	(-0.002,0.012)	.1294



A two-sample *t*-test was performed to evaluate differences in FAS and MRSI between high vs. low Gleason (3+3, 3+4) tumors. There were no significant differences in FAS (tumor $p=.3611$; benign $p=.5230$) or MRSI (tumor $p=.7729$; benign $p=.3401$) between high vs. low Gleason tumors.

Table 6. Comparison between high vs. low Gleason tumors

Comparison	Gleason Group	N	Mean (SE)	95% CI of Difference	P value
FAS Tumor	High	11	217.3 (23.2)	(-80.9, 30.7)	.3611
	Low	14	192.1 (158.6)		
FAS Benign	High	12	122.5 (44.5)	(-45.9, 24.0)	.5230
	Low	13	111.5 (40.0)		
MRSI Tumor	High	12	0.027 (0.007)	(-0.018,0.024)	.7729
	Low	15	0.030 (0.007)		
MRSI Benign	High	12	0.019 (0.005)	(-0.009,0.024)	.3401
	Low	15	0.026 (0.006)		

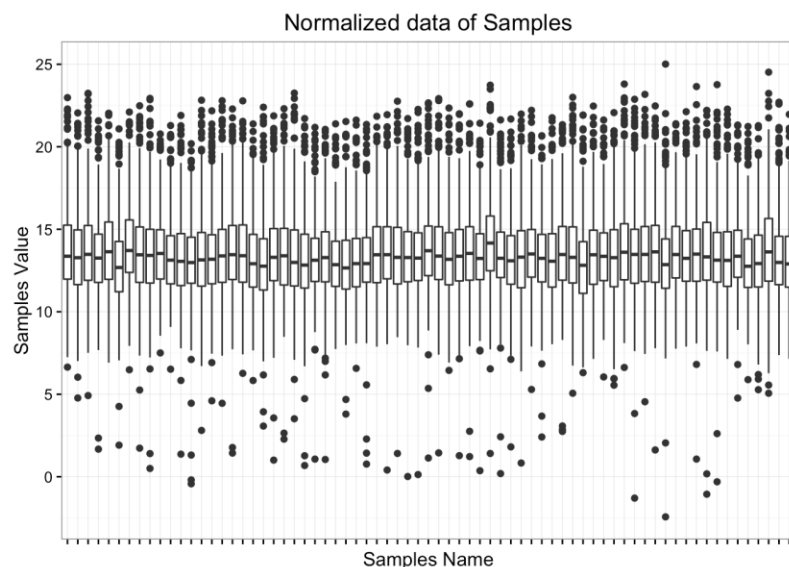


Aim3: Comparison of metabolomic data between benign vs. tumor samples, high vs. low Gleason tumors, metabolomics vs. FAS, metabolomics vs. MRSI in benign and tumor tissues.

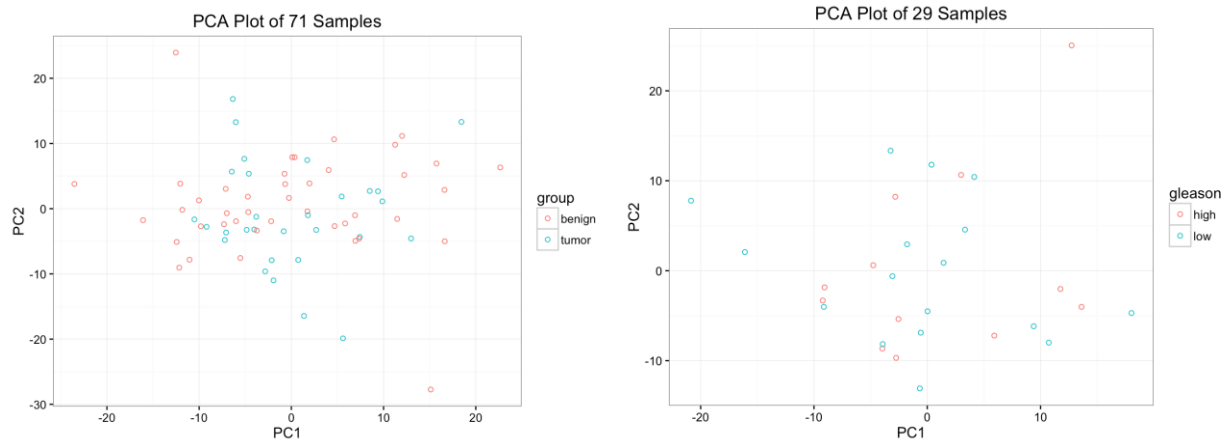
There were a total of 71 samples, and 469 metabolites were interrogated simultaneously. The data were pre-processed and normalized using the R package 'metabolomics' using the "nomis" method, which uses optimal selection of multiple internal standards [Sysi-Aho M, Katajamaa M, Yetukuri L, Oresic M (2007). Normalization method for metabolomics data using optimal selection of multiple internal standards. BMC Bioinformatics 8(1): 93.].

See the documentaion on the R metabolomics package:

<https://cran.r-project.org/web/packages/metabolomics/metabolomics.pdf>



Exploratory data analyses were performed to evaluate general data quality and consistency. The Principal Component Analysis (PCA) was performed to assess general clustering of samples by tissue types or disease groups.



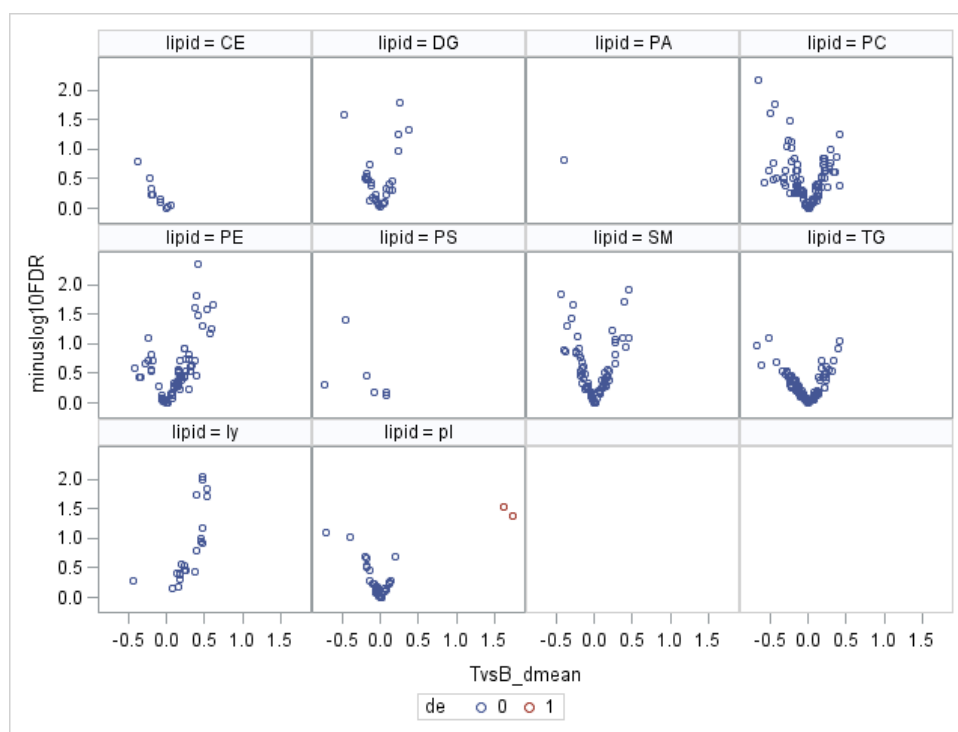
Aim 3a: Metabolites differentially expressed between tumors and benign tissues

Mixed effects models were used to determine which metabolites were differentially expressed between tumors vs. benign samples. Significant differential expression (de) was defined as having at least two-fold difference between tumor and benign tissues and FDR adjusted p value of $<.20$. Two metabolites were identified as differentially expressed:

plasmenyl-PE 36:2; [M+Na]⁺@6.83

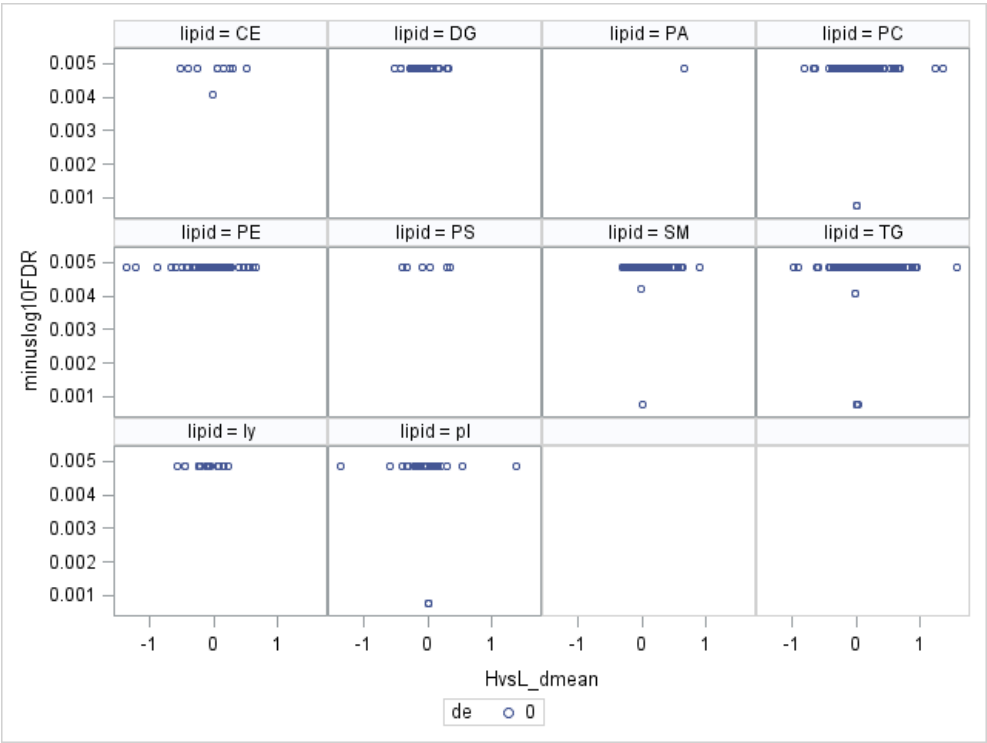
plasmenyl-PE 38:5; [M+H]⁺@5.11

A volcano plot below shows $-\log_{10}(\text{FDR adjusted } p \text{ value})$ vs. mean difference between tumors and benign tissues by 10 different lipid classes. Each dot represents a specific metabolite. A red dot indicates a differentially expressed (de) metabolite.



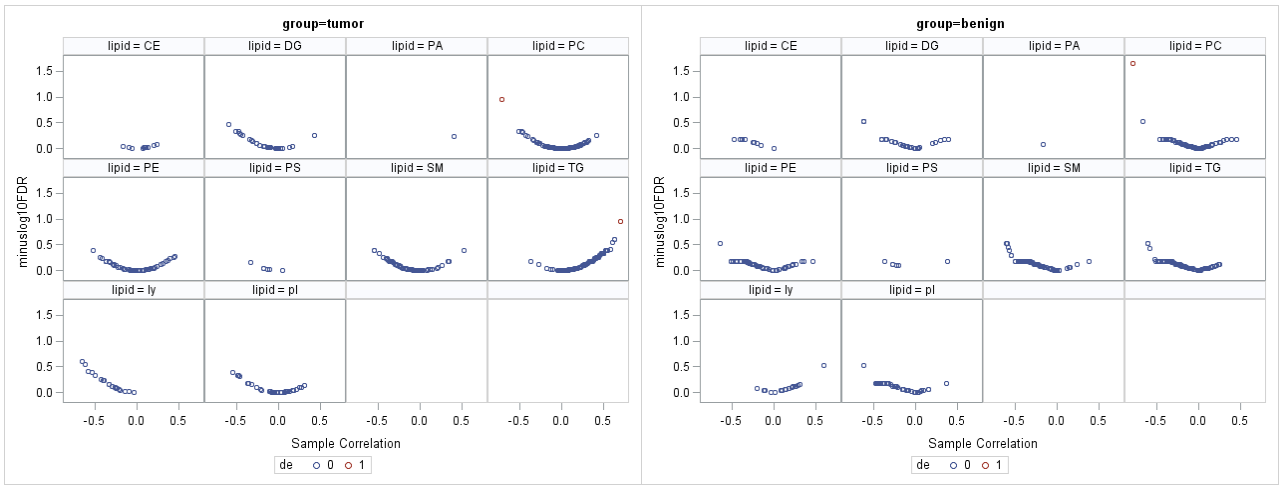
Aim 3b: Metabolites that were differentially expressed between high vs. low Gleason tumors

Generalized linear models were performed to determine which metabolites were differentially expressed between high vs. low Gleason tumors. None met the differential expression (de) criteria.



Aim 3c: Metabolites that were significantly associated with FAS

Spearman correlation coefficients were computed between FAS and each metabolite. Significant differential expression (de) was defined as having a correlation coefficient of at least ± 0.30 and FDR p value $< .20$.

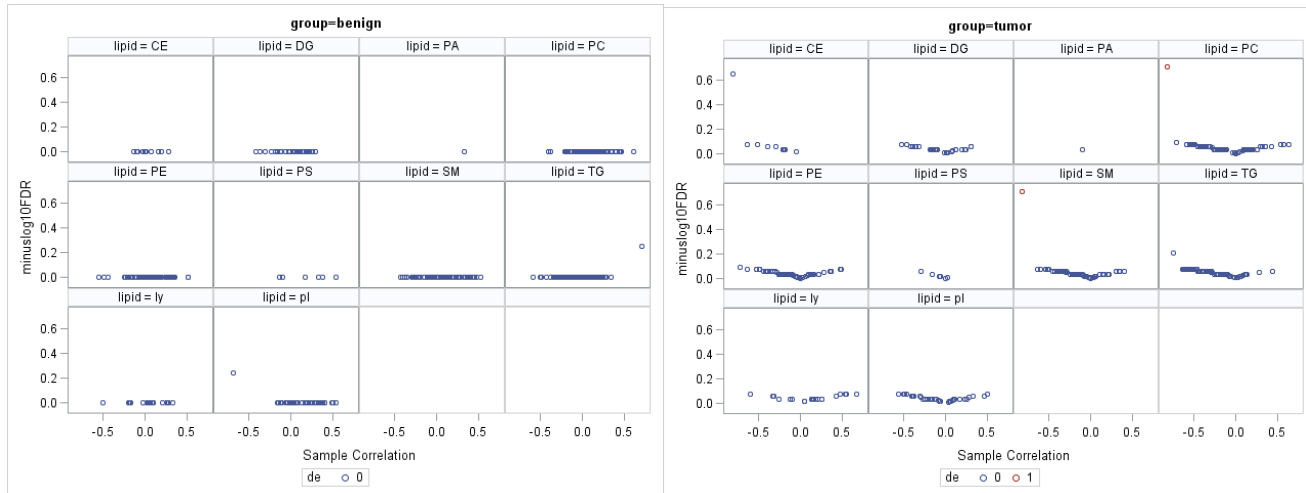


There were three metabolites that met the criteria:

group	metabolite	Corr	pValue	fdr_p	lipid	minuslog10FDR
benign	PC 44:5; [M+H] ⁺ @7.57	-0.78148	<.0001	0.02205	PC	1.65651
tumor	PC 36:4; [M+H] ⁺ @3.82	-0.70375	0.0005	0.10943	PC	0.96086
tumor	TG 56:8; [M+NH ₄] ⁺ @9.80	0.70286	0.0005	0.10943	TG	0.96086

Aim 3d: Metabolites that were significantly associated with MRSI in tumors

Spearman correlation coefficients were computed between MRSI and each metabolite. Significant differential expression (de) was defined as having a correlation coefficient of at least ± 0.30 and FDR p value < .20. There were two metabolites that met the criteria.



group	Metabolite	Corr	pValue	fdr_p	lipid	minuslog10FDR
tumor	PC 42:4; [M+H] ⁺ @7.54	-0.82727	0.0009	0.19383	PC	0.71259
tumor	SM 30:1; [M] ⁺ @3.83	-0.82727	0.0009	0.19383	SM	0.71259

CONCLUSION:

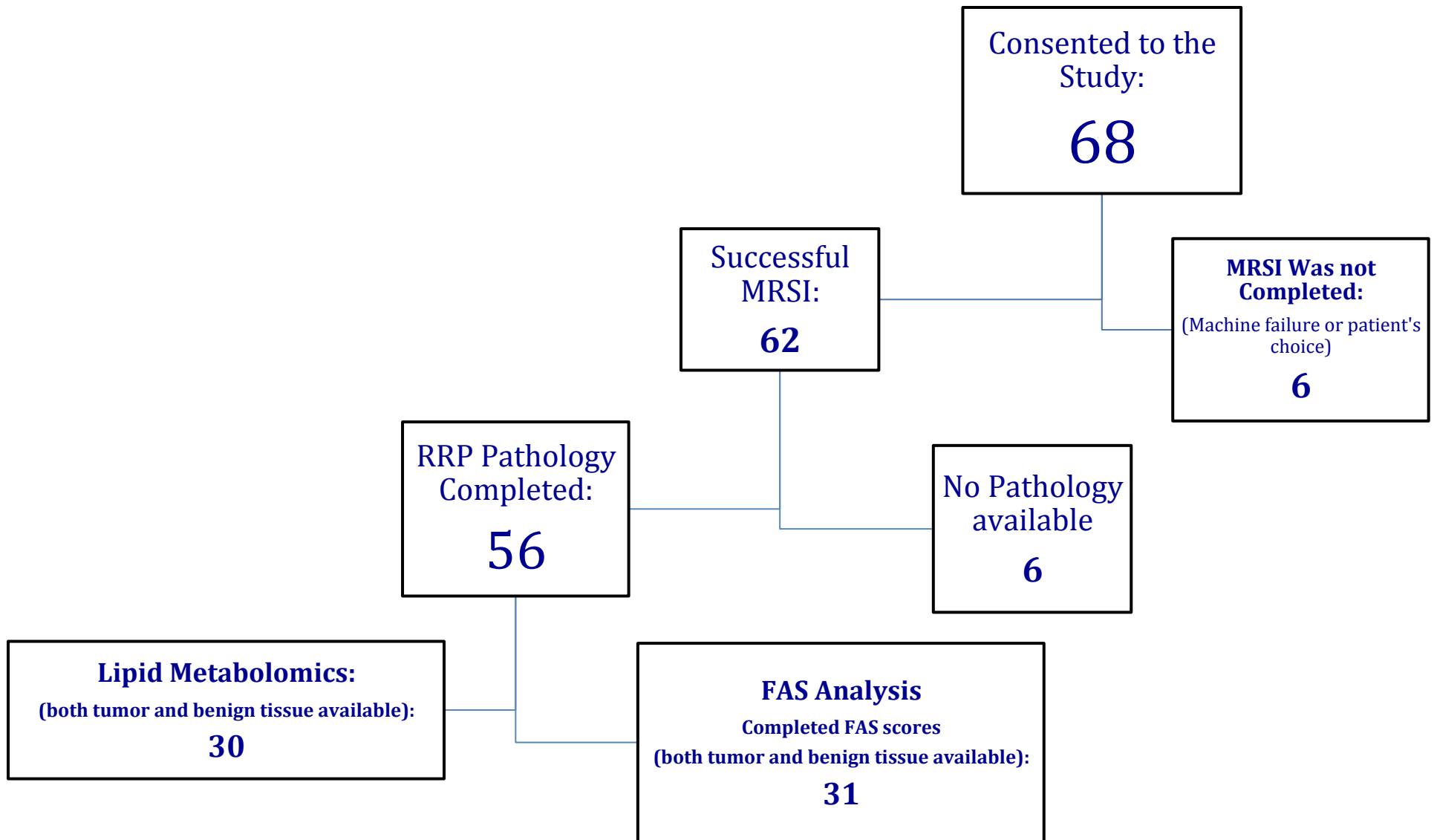
Even though the team was aware of the potential challenges prior to the commencement of the prostate MRSI data acquisition, the actual challenges presented during each individual study still played a major confounding factor in the data collection. As such, the number of MRSI data sets included in the final data analysis is about half of the total subjects enrolled in the study. We summarize these “unsuccessful” cases into three major categories, which we hope will provide useful information for similar effort in the future: 1) 11 subjects with no MRI data (due to screening failure, subjects withdraw, and scanner glitches). One common issue here is that clinical prostatectomy procedures scheduled following the MRSI often excluded the chance for rescheduling of the MRI study procedure; 2) 9 cases where no convincing suspicious foci were visible on MRIs. Besides potential false positive lesion that can present on MRIs, these “false negative” cases also present as a limitation for using MRI to detect prostate cancer; 3) 16 incomplete MRSI data sets. One common factor for this sub-group of patients with advanced prostate cancer is that MR imaging time is generally too long. Some subjects requested to stop the scan before data collection was completed as planned.

REFERENCES:

X. Li, J. Shannon, M. G. Garzotto, C. Amling, W. J. Woodward, G. Thomas, E. Dacey, X. Wang, P. Farris, W. Stoller, A. M. Acevedo, A. Palma, M. Sammi, W. D. Rooney, F. V. Coakley, J. Purnell, “Intraprostatic Lipid Spectroscopic Imaging of the Prostate Cancer”, Proc. Intl. Soc. Mag. Reson. Med. 23, 3838 (2015).

APPENDICES:

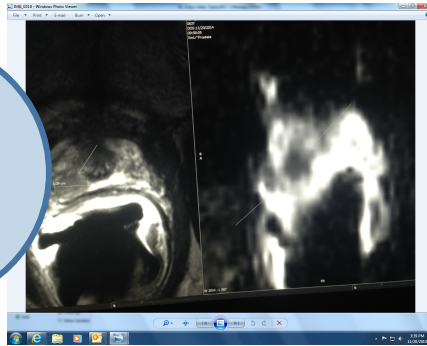
1. MRSI Subject Status Flowchart
2. MRSI Research specimen collection Standard Operating Procedure



Informed Consent & Safety Checklist Completed

MRSI Procedure

1. MRSI Image Collected (Dr. Li)



2. Clinical PiRADs Score Assigned (Dr. Coakley)

3. Measurements Determined (Dr. Li)

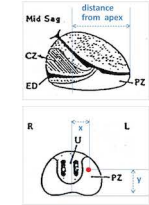
Imaging prostatic lipids to distinguish aggressive prostate cancer – REPORT FORM
Subject #LIPID-029
MRSI Date: 09/22/2014
Fergus' read: 09/23/2014
Xin's measurements: 09/25/2014

MRSI Prostate Image Measurements: slice 1	
MRSI Date: 9/22/2014	
Distance from apex:	37 mm
Distance from left-right mid line (L/R):	L 16 mm
Distance from posterior margin (A-P):	9 mm
Largest cross-section measure (Ax by Dy):	45.7 mm by 40.7 mm

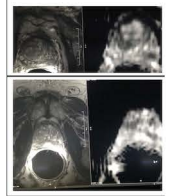
MRSI Prostate Image Measurements: slice 2	
MRSI Date: 9/22/2014	
Distance from apex:	32 mm
Distance from left-right mid line (L/R):	L 17 mm
Distance from posterior margin (A-P):	8 mm
Largest cross-section measure (Ax by Dy):	48.7 mm by 38.5 mm

MRSI Prostate Image Measurements: slice 3	
MRSI Date: 9/22/2014	
Distance from apex:	22 mm
Distance from left-right mid line (L/R):	L 15 mm
Distance from posterior margin (A-P):	25 mm
Largest cross-section measure (Ax by Dy):	49.6 mm by 33.2 mm

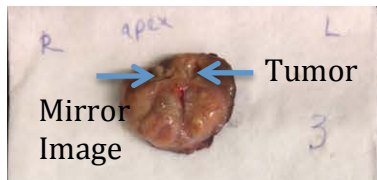
P.S:
Largest prostate dimensions on MRI:
AP: 40.7 mm
HF: 47.8 mm
LR: 49.6 mm
HF: roughly apex to base, from T2* coronal view.



DESCRIPTION on cartoon FROM FERGUS:
Interesting, looks like kind of spotty organ-confined disease on the left side, two biggest foci at the left base and apex (see attached). Base



Example (1 of 3 slices):



Each tissue sample is divided 3 ways

H&E Slides created with cells from cut

Research Pathology Processing:

- Specimen collection per MRSI measurements (pathology resident)
- Mirror image (assumed benign) specimen collected
- Documentation of research cuts (residents & research coordinators)

Some tissue (same cut) is Flash Frozen & stored at -80°F

Piece of tissue (same cut) placed in formalin

TOTAL FOR EACH PROSTATE SLICE TUMOR

- 2 slides
 - 1 flash frozen
 - 1 formalin
- ### MIRROR IMAGE
- 2 slides
 - 1 flash frozen
 - 1 formalin

Research Specimen Processing by Dr. George Thomas:

1

Review of H&E slides prepared by pathology. Tissue containing highest tumor content is chosen and matched to benign sample.

2

Tissue containing largest tumor selected and sent to HSR for FFPE; tissue combined to one slide for FAS IHC

3

FAS slides reviewed

4

Flash-frozen tissue samples - selected based on frozen section slides (i.e. tumor-normal pairs) - sent to Baylor